Transplantable Liver Organoids Made from Only Three Ingredients

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Liver cell therapies using induced pluripotent stem cells (iPSCs) are in development. A recent paper in *Nature* by Takebe et al. expands the range of liver diseases that could be treated with iPSC-derived hepatocytes by combining them with endothelial and stromal cells to generate organoids that survive and function extrahepatically.

Possibly the greatest potential of human induced pluripotent stem cells (iPSCs) lies in autologous cell therapies requiring no or little immune suppression. The liver is a particularly promising target for cell therapy because of its inherent ability to integrate newly formed cells, including hepatocytes, which provide most of the liver's functions. While current protocols for generating hepatocytes from iPSCs (iPSC-Heps) yield incompletely differentiated cells, there is steady progress toward obtaining iPSC-Heps that are equivalent to primary human hepatocytes in both function and ability to proliferate. However, clinical experience with primary human hepatocyte transplantation suggests that using fully functional cells and protecting them from immune rejection is not a guarantee for successful liver cell therapy (Puppi et al., 2012). To be therapeutically effective, transplanted cells also need to efficiently engraft and survive long term—factors that are impacted by the liver microenvironment and thus the underlying disease. Unfortunately, most liver diseases are associated with fibrosis, which in its most advanced form, cirrhosis, impairs engraftment, survival, and function of transplanted hepatocytes (Liu et al., 2012). Therefore, extrahepatic transplantation sites such as lymph nodes are currently being pursued to realize the full potential of liver cell therapy (Hoppo et al., 2011).

A promising alternative approach has now been reported by Takebe et al. (2013). Instead of introducing iPSC-Heps into a hostile or foreign microenvironment, the authors delivered the cells to extrahepatic sites as self-contained organoids mimicking embryonic liver (Figure 1). They started by generating hepatic endoderm cells from human iPSCs (iPSC-HECs) using standard protocols. Because stromal and endothelial cells provide essential cues for liver development (Zaret and Grompe, 2008), the authors reasoned that adding human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (MSCs) to the iPSC-HECs could assist in proper differentiation. Surprisingly, within a day, the authors observed that the three cell types began to self-assemble into 3D organoids in which the MSCs provided structural support and the HUVECs organized into a loose web. This microenvironment promoted hepatocyte differentiation of the iPSC-HECs, eventually producing iPSC-Heps that were still immature, but expressed certain liver functions at higher levels than iPSC-Heps generated in 2D cultures.

The organoids could be detached from the culture matrix, which facilitated transplanting them as intact structures. Initially, Takebe et al. placed single organoids on the brains of immune-deficient mice and monitored them through cranial windows. Only 2 days after transplantation, the organoids exhibited HUVEC-derived blood vessels that were connected to the host circulation and rapidly formed a complex vascular network. This finding of spontaneous organization of HUVECs into functional blood vessels in vivo is reminiscent of previous studies (Koike et al., 2004), which also established the importance of MSCs as a source of pericytes promoting vessel stability.

Analysis of the organoids 2 months after transplantation revealed that they were viable and had undergone further maturation. Histologically, organoids resembled adult liver in that iPSC-Heps had assumed a cuboidal shape, were organized in cords, and had formed bile canaliculi with adjacent iPSC-Heps. However, because there were no bile ducts, bile produced by the iPSC-Heps was likely released into the circulation, which may be tolerated if the recipient's native liver retains excretory function (Hoppo et al., 2011). Integrated organoids allowed analysis of human-specific drug metabolism, suggesting further differentiation of iPSC-Heps in vivo. However, iPSC-Heps were not fully differentiated, as evidenced by lower albumin secretion and lower expression of hepatocyte-specific CYP450 enzymes than previously reported for primary human hepatocytes (Azuma et al., 2007). The authors attributed this deficiency to delayed differentiation of human cells in mice, a possibility that could be addressed by following transplanted organoids for more time. Alternatively, the iPSC line or the differentiation protocol used may not allow completion of hepatocyte differentiation in vivo, or iPSC-Heps may require exposure to the specialized stromal and endothelial cells or the venous-arterial blood mix found in the normal liver for full differentiation.

Takebe et al. also established transplantation of numerous organoids into extrahepatic sites, namely the space

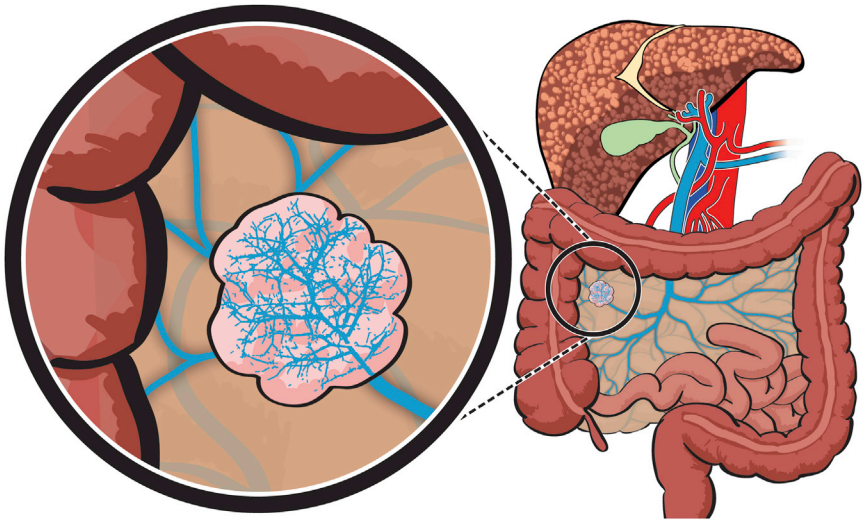

Figure 1. Transplantation of Liver Organoids into Extrahepatic Sites such as the Mesentery as a Potential Therapy for Liver Cirrhosis
Arteries are shown in red, veins in blue, and biliary structures in green. Only the veins of the mesentery are shown.

under the kidney capsule and, with an eye toward clinical translation, the mesentery. They showed that transplanting 12 organoids (corresponding to ~1% of a mouse's hepatocyte mass) on the mesentery was effective in protecting mice from subacute liver failure. Because the human liver contains ~1,000 times more hepatocytes than the mouse liver, translating these promising results into a therapy for patients with liver disease will depend on whether the approach can be significantly scaled up. As first evidence for the feasibility of scaling, Takebe et al. found spontaneous proliferation of iPSC-Heps in transplanted organoids, resulting in ~20-fold expansion. iPSC-Hep proliferation could be further increased by 2/3 partial hepatectomy, which suggests that hepatocyte growth factors could be used for noninvasive posttransplant expansion. As an alternative to transplanting a large number of organoids and expanding them in vivo, it may be possible to assemble numerous organoids in a decellularized liver matrix in vitro to generate a single transplantable structure that could be connected to both venous and arterial circulation (Uygun et al., 2010). Recellularizing natural liver scaffolds has proven to be difficult, and using organoids as self-connecting building blocks may facilitate the formation of a functional vascular network, particularly if flow is provided by perfusing the scaffold through the portal vein.

Finally, although not investigated in the study by Takebe et al., generating entirely autologous organoids requiring no or little immune suppression appears feasible. To do so may simply require following established protocols for the directed differentiation of human embryonic stem cells into endothelial cells and MSCs (Gruenloh et al., 2011; Wang et al., 2007).

The study by Takebe et al. has implications beyond liver cell therapy. The ease with which liver organoids could be generated and engrafted serves to remind us that cells normally work in concert with other cells, and that they carry information on how to come together as a viable tissue, properties to be considered and harnessed for regenerative medicine.

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REFERENCES

Azuma, H., Paulk, N., Ranade, A., Dorrell, C., Al-Dhalimy, M., Ellis, E., Strom, S., Kay, M.A., Finegold, M., and Grompe, M. (2007). Nat. Biotechnol. *25*, 903–910.

Gruenloh, W., Kambal, A., Sondergaard, C., McGee, J., Nacey, C., Kalomoiris, S., Pepper, K., Olson, S., Fierro, F., and Nolta, J.A. (2011). Tissue Eng. Part A *17*, 1517–1525.

Hoppo, T., Komori, J., Manohar, R., Stolz, D.B., and Lagasse, E. (2011). Gastroenterology *140*, 656–666, e652.

Koike, N., Fukumura, D., Gralla, O., Au, P., Schechner, J.S., and Jain, R.K. (2004). Nature *428*, 138–139.

Liu, L., Yannam, G.R., Nishikawa, T., Yamamoto, T., Basma, H., Ito, R., Nagaya, M., Dutta-Moscato, J., Stolz, D.B., Duan, F., et al. (2012). Hepatology *55*, 1529–1539.

Puppi, J., Strom, S.C., Hughes, R.D., Bansal, S., Castell, J.V., Dagher, I., Ellis, E.C., Nowak, G., Ericzon, B.G., Fox, I.J., et al. (2012). Cell Transplant. *21*, 1–10.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.R., Ueno, Y., Zheng, Y.W., Koike, N., et al. (2013). Nature, in press. Published online July 3, 2013. http://dx.doi.org/10.1038/nature12271.

Uygun, B.E., Soto-Gutierrez, A., Yagi, H., Izamis, M.L., Guzzardi, M.A., Shulman, C., Milwid, J., Kobayashi, N., Tilles, A., Berthiaume, F., et al. (2010). Nat. Med. *16*, 814–820.

Wang, Z.Z., Au, P., Chen, T., Shao, Y., Daheron, L.M., Bai, H., Arzigian, M., Fukumura, D., Jain, R.K., and Scadden, D.T. (2007). Nat. Biotechnol. *25*, 317–318.

Zaret, K.S., and Grompe, M. (2008). Science *322*, 1490–1494.